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Axonal outgrowth on nano-imprinted patterns

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Abstract

Nanotechnology has provided methods to fabricate surface patterns with features down to a few nm. If cells or cell processes exhibit contact guidance in response to such small patterns is an interesting question and could be pertinent for many applications. In the present study we investigated if axonal outgrowth was affected by nano-printed patterns in polymethylmethacrylate (PMMA)-covered silicon chips. To this end adult mouse sympathetic and sensory ganglia were mounted in Matrigel® on the chips close to the nano-patterns. The patterns consisted of parallel grooves with depths of 300 nm and varying widths of 100–400 nm. The distance between two adjacent grooves was 100–1600 nm. The chips were cultured in medium containing 25 ng/ml of nerve growth factor to stimulate axonal outgrowth. After 1 week of incubation, axonal outgrowth was investigated by immunocytochemistry or scanning electron microscopy. Axons displayed contact guidance on all patterns. Furthermore, we found that the nerve cell processes preferred to grow on ridge edges and elevations in the patterns rather than in grooves, a seemingly claustrophobic behavior. We conclude that axons of peripheral neurons might be guided by nanopatterns on PMMA when the lateral features are 100 nm or larger. The present results can be utilized for nerve regenerating scaffolds or the construction of a stable, high-resolution electronic interface to neurons, which is required for future brain machine interfaces.

Keywords: Cell culture; Nanotopography; Nerve guide; Nerve regeneration; Polymethylmethacrylate

1. Introduction

Extensive efforts are made to construct a junction between neurons and electronic chips i.e. a brain machine interface (BMI) [1,2]. The potential impact of such devices is enormous since they can be used to compensate for both sensory and motor deficits in the nervous systems e.g. they could be used to restore vision, hearing and motor impairments as well as impaired autonomic functions. This feat is however not trivial since the number of nerve cell processes and neurons are counted in the millions and efficient neuro-electronic junctions must be small. Thus they must have a high-spatial resolution; here nanotechnology may offer a solution. In this study we tested whether neuronal processes, axons, could be guided by nanopatterns (grooves and ridges), and if these nanopatterns possess the spatial resolution required to contact thousands of nerve fibers on a small silicon/polymer-based chip surface.

For single cells, the cellular response to alterations in surface topography, down to the micrometer scale has been well documented especially for grooved topography [3–6]. Most cells follow the discontinuities of grooves and ridges, and attain an elongated shape due to surface-induced rearrangements of the cytoskeleton [4]. Grooved surfaces also induce changes in transcription and the up and down regulation of several genes [7], but the explicit mechanism for cell guidance has yet to be clarified. The development of techniques to produce surface structures in the nanometer range has revealed

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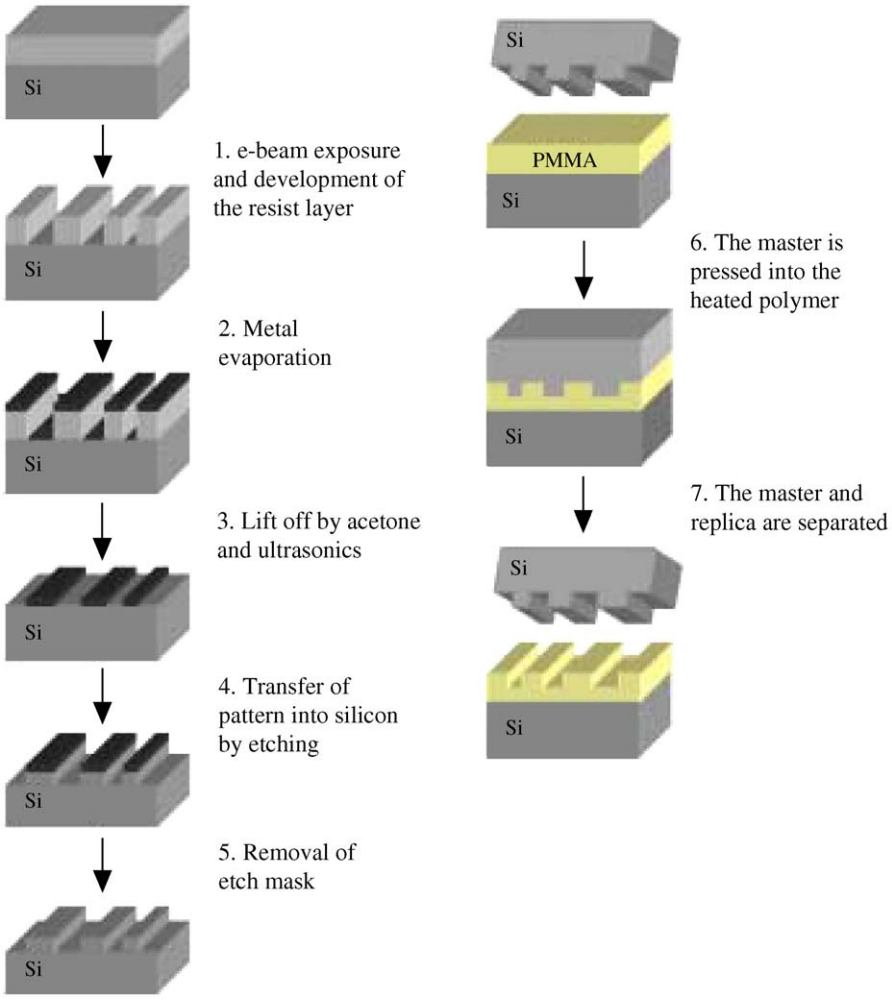

Fig. 1. Master fabrication by electron beam lithography (EBL), left column and nanoimprint lithography (NIL), right column: EBL: First a silicon wafer is spin coated with a lift off layer (LOL). The resist is exposed with an e-beam and developed to give the desired pattern. A thin layer of chrome is evaporated onto the pattern. In the lift-off step, the LOL dissolves and leaves a chrome etch mask behind. After etching in KOH, the chrome is removed and the silicon master is ready. NIL: The silicon master is pressed (50 bar) into a layer of polymethylmethacrylate (PMMA) under heating (180 °C). When cooled, the master is peeled away and the imprinted pattern remains, supported by the silicon chip.

that cells also respond to such nanostructures. Macrophage-like cells can react to steps in the nanometer range [8]. Endothelial and fibroblast cells are sensitive to patterns with features down to 10 nm [4,9,10]. Furthermore the importance of symmetry or discontinuity of the nanometer patterns was pointed out [4]. However, the response of neurons and their axons, to nanopatterned substrata, have not been studied in detail. Rajnicek et al [11] reported that central nervous system (CNS) neurites were guided by shallow (14 nm deep and 1 μm wide) grooves. Furthermore, contact guidance for chicken dorsal root ganglion (DRG) neurons on single scratches (0.1–0.2 μm wide), was reported by Stepien and coworkers [12]. Fibroblast filopodia have also been found to respond to nanotopographies [10,13], whether or not, axonal or growth cone filopodia also respond to submicron structures have hitherto not been determined.

In this study we used nanoimprint lithography (NIL), on polymer (polymethylmethacrylate—PMMA) covered silicon wafers (Fig. 1), to test the reaction of neuronal processes to grooves and ridges smaller than 1 μm. The purpose of the study was to investigate if axons could be guided by nanometer sized patterns. The study of axonal guidance differs from studies of single cell guidance. When dealing with guided single cells, the elongation of the cell gives a clear axis indicating the guiding direction. In the case of axons, the length scale is much different and the axons have a wandering behavior. Even if an axon shows clear contact guidance, it may change to an adjacent ridge. This makes it difficult to compare the guiding properties of different patterns and a more macroscopic method must be adopted (Figs. 5 and 6). In this study we present a simple fast Fourier transform (FFT) analysis in order to quantify the guidance on different patterns.